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Optimisation of crude extract for preservation and sensory improvement in paneer quality

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Abstract
The study evaluated the efficacy of crude extracts derived from a nisin-producing strain of Lactococcus lactis ssp. lactis as a bio-preservative to enhance the shelf life of paneer. The investigation employed microbiological analyses and sensory evaluations to determine the optimal concentration of the crude extract. Two treatment groups were established: one with a 50% crude extract (B1) and another with a 100% crude extract (B2). The results indicated that the 100% crude extract (B2) was the most effective treatment, demonstrating significantly lower bacterial, yeast, and mould counts compared to B1. Microbiological assessments revealed that paneer treated with B2 maintained superior microbiological quality throughout the storage period. Total plate counts in the treatment group remained significantly lower (p<0.01) than in the control group, with a mean of 2.60 and 2.28 for B1 and B2 respectively by day 6, continuing this trend until day 12. While yeast and mould counts remained absent in the control group until day 9, colonies were present within the limits suggested by FSSAI in the treatment group starting from day 6.
Sensory evaluations showed no significant differences (p<0.05) in appearance and colour between the control and treatment groups; however, scores for body, texture, and flavour were superior in the treatment group. For odour, aftertaste, and overall acceptability, control scores were higher after day 9. This study demonstrates that bio-preservation using crude extracts from Lactococcus lactis provides effective preservative action in paneer, maintaining its quality for up to 9 days of storage.

Keywords: Paneer, crude extract, anti-microbial activity, preservation

1. Introduction
Paneer is a traditional soft cheese produced through the heat and acid coagulation of milk. Due to its high moisture content, paneer is particularly susceptible to microbial spoilage and subsequent biochemical deterioration. Various preservation methods, including chemical preservatives, low-temperature storage and hurdle technology, have been explored to extend the shelf life of paneer. However, the use of natural food preservatives offers a promising alternative to chemical methods, alleviating consumer concerns about potential health risks. Bio-functional components derived from fermented milk can serve as effective bio-preservatives, enhancing the shelf life of perishable food products. The shelf life of paneer is influenced by multiple factors, including microbial load, storage conditions and the presence of preservatives. Microbial spoilage poses significant challenges, leading to undesirable flavours, textures and safety concerns. Common spoilage microorganisms, such as bacteria, yeasts and moulds, can proliferate rapidly under inappropriate storage conditions. Therefore, maintaining optimal shelf life is essential for consumer satisfaction and reducing food waste. Traditional methods for extending paneer's shelf life primarily involve refrigeration; however, these methods may not sufficiently prevent spoilage over extended periods. Consequently, the incorporation of natural preservatives, such as lactic acid bacteria (LAB) and their metabolites, has garnered increasing attention. These bio-preservatives not only inhibit microbial growth but also enhance the sensory qualities of paneer. Understanding the factors influencing the shelf life of paneer and exploring innovative preservation techniques are crucial for improving product quality and safety. This study investigates the potential of crude extracts from nisin-producing strains of Lactococcus lactis as a natural bio-preservative, aiming to enhance the

shelf life and overall quality of paneer. This approach offers a promising solution to meet consumer demands for fresh and safe dairy products.

2. Materials and Methods

2.1 Procurement of lactic culture

The lactic acid bacteria Lactococcus lactis ssp. lactis 94 was obtained from the National Collection of Dairy Cultures (NCDC) at the National Dairy Research Institute in Karnal. This culture was subsequently propagated in skim milk and stored at 4 °C, with periodic assessments conducted to evaluate its viability.

2.2 Preparation of paneer

Pasteurised cow milk was obtained from the Dairy Plant at Kerala Veterinary and Animal Sciences University, Mannuthy. Paneer was prepared following the method outlined by Khan and Pal (2011) [6]. The milk was heated to 90°C and then rapidly cooled to 85 °C. Coagulation was induced by adding a 2% citric acid solution at 85 °C while stirring gently. The coagulated mixture was allowed to rest in the whey for five minutes before being strained through muslin cloth. The cloth containing the paneer was tied and pressed in a hoop for 15-20 minutes, after which the pressed paneer was immersed in chilled water for 30 minutes. Finally, the paneer was cut into uniform 5 cm^3 blocks.

2.3 Optimisation of the bio-preservative dose

2.3.1 Propagation of Cultures

Lyophilized cultures of Lactococcus lactis ssp. lactis (94-NDRI nisin-producing strain) were sourced from the National Collection of Dairy Cultures at the National Dairy Research Institute in Karnal. Initially, these cultures were propagated in 3 mL of skim milk, and the mother culture was subsequently propagated weekly in 10 mL of skim milk. The viability of the culture was assessed and a glycerol stock was prepared and stored at -20°C for long-term use.

2.3.2 Preparation of curd using propagated culture

Curd was prepared by adding two per cent of starter culture inoculum from the propagated culture to cow milk and incubated at 37 ºC for 24 h.

2.3.3 Preparation of crude extract (CE) from curd

The crude extract (CE) was prepared according to the method described by Das *et al*. (2018) [4] with minor modifications. For each batch, 40 mL of curd sample was thoroughly homogenized in a sterile glass beaker for three to five minutes. The homogenized mixture was then centrifuged at 5000 rpm for 20 minutes. After centrifugation, 20 mL of the supernatant was collected, and the thin layer of fat on top was removed. The supernatant was filtered first through Whatman No. 1 filter paper and then through a syringe filter (0.2-micron, Super Membrane, Low Protein Binding, Non-Pyrogenic, PN 4612). The resulting filtrate constituted the crude extract (CE), which was aliquoted and stored at -20°C for future use.

2.3.4 Preparation of McFarland Standard

The turbidity standard solution was prepared by adding 0.5mL of 0.048mol/L $BaCl_2$ to 99.5 mL of 0.18 mol/L H_2SO_4 (one per cent w/v). This solution was equal to half the density of No.1 McFarland standard solution. This solution was taken into glass tube, sealed tightly and kept in the dark, at room temperature for further use. The tube was vigorously agitated just before each use.

2.3.5 Preparation and Standardisation of Inoculum

Three to four isolated colonies were selected from a pure culture and transferred into sterile nutrient broth, where they were incubated at 37 °C for 12 hours. The turbidity of the culture was then adjusted to match the 0.5 McFarland standard solution. If the broth culture appeared too turbid, it was diluted with additional nutrient broth; conversely, if the turbidity was too low, the culture was incubated longer until the desired turbidity was achieved.

2.3.6 Test procedure

Antibacterial activity of crude extract was determined by agar disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI, 2020). [3] A sterile cotton swab on a wooden applicator was dipped into the standardised inoculum of E. coli culture (0.5 McFarland) and excess fluid was removed by pressing and rotating the swab against the side of the tube above the suspension. The entire agar surface of the MHA agar plates was spread with the swab three times, turning the plate at 60º angle between each spreading to ensure even distribution. Under aseptic conditions, five sterile discs were placed on MHA agar plates using sterile forceps with distance of 24 mm (centre to centre). A sterile pipette was used to place 10 µL of various concentrations of crude extract into each disc. The plates were then incubated at 37ºC for 24 h. The diameter of the zone of complete inhibition was measured (Bauer *et al*., 1966) [1]. The experiment was repeated thrice.

2.3.7 Determination of dose of crude extract

Prepared paneer cubes were dipped in two different concentrations of crude extract prepared for 15 min. (Das *et al*., 2018) [4] and packed in LDPE pouches, stored at 4±1 °C. Quality of each sample was assessed based on sensory and microbiological characteristics at 0^{th}, 3^{rd}, 6^{th}, 9^{th} and 12^{th} day of storage for texture, flavour, and aroma to identify the optimal dosage, which was used for further studies.

2.4 Assessment of total plate count in paneer sample

Total viable count of each sample was estimated by pour plate technique, as described by Wehr and Frank (2004) [11]. To one millilitre sample taken in petridish about 15-20 mL sterile molten standard plate count agar maintained at 45 ºC was poured and mixed by gentle clockwise and anticlockwise movement. After solidification of media samples were incubated at 37 ºC for 24 h. At the end of incubation period, colonies were counted and expressed as $\log_{10}$ CFU/g.

2.5 Assessment of total Yeast and Mould count in paneer sample

Yeast and Mold Count of paneer samples were determined according to the procedure laid out by the Bureau of Indian Standards (IS: 18, 1980) [2]. To one millilitre sample taken in petri-dish about 15-20 mL sterile molten potato dextrose agar and maintained at 45ºC was poured and mixed by gentle clockwise and anticlockwise movement. After solidification of media samples were incubated at 25ºC for 72 h. At the end of incubation period, colonies were counted and expressed as $\log_{10}$ CFU/g.

2.6 Sensory analysis

Paneer samples were evaluated for their sensory characteristics, including appearance and colour, body and texture, flavour, odour, after taste, as well as overall acceptability scores, following the method proposed by Ranganna (2008) [10] The average scores were determined based on evaluations from six panel members.

2.7 Statical analysis

Six replications were performed, and the data collected were analysed using SPSS version 24.0. To identify significant differences among various parameters, both one-way ANOVA and repeated measures ANOVA were conducted. Non-parametric tests were employed for evaluating the sensory attributes of different paneer samples.

3. Results and Discussion

3.1 Optimisation of level of bio-preservative for paneer

Paneer cubes were treated with two concentrations of crude extract derived from curd: 50% (B1) and 100% (B2), and then packed in LDPE pouches. The optimal concentration was determined through sensory and microbiological evaluations. Sensory assessments utilized a nine-point hedonic scale to evaluate key attributes such as appearance and colour, body and texture, flavour, odour, aftertaste, and overall acceptability. These evaluations helped to identify the most effective treatment for improving the quality and shelf life of paneer.

3.2 Antimicrobial activity of crude extract

The antimicrobial activity of the crude extract was demonstrated by a clear 5 mm zone of inhibition against E. coli on Mueller-Hinton Agar (MHA) plates, indicating its effectiveness in preventing E. coli growth. This suggests its potential as a bio-preservative in paneer, enhancing food safety and extending shelf life by reducing microbial contamination. These findings align with research by Kanver *et al*. (1995), who reported high microbial loads, particularly lactic acid bacteria (LAB), in curd samples. LAB are well-known for their role in dairy fermentation and their ability to produce antimicrobial compounds such as organic acids, hydrogen peroxide, and bacteriocins. These bacteriocins are small peptides synthesised by ribosomes that possess strong bactericidal properties, allowing them to inhibit or kill a variety of harmful microorganisms, including pathogens like E. coli.

3.3 Total plate count of control and paneer dipped in different concentrations of crude extract

The bio-preservation doses were assessed based on total plate counts, as illustrated in Table 1. Until day 3, no bacterial growth was detected in either the control or the bio-preservative-treated samples (B1 and B2). By day 6, bacterial counts were 2.85 log CFU/g in the control, 2.60 log CFU/g in B1, and 2.28 log CFU/g in B2. On day 9, these counts increased, with B2 consistently showing the lowest growth at 2.95 log CFU/g compared to the control and B1. By day 12, B2 demonstrated the most significant reduction in bacterial growth, remaining well below control levels and within FSSAI limits. This indicates that B2 was the most effective at controlling microbial growth and extending shelf life. Mabrouk *et al*. (2014) [8] reported that LAB displayed antimicrobial activities against S. aureus, E. coli, P. aeruginosa, L. monocytogenes, and B. cereus, aligning with the current study's findings. By reducing microbial contamination, the extract can greatly enhance food safety and prolong the shelf life of paneer, thereby mitigating spoilage and the risk of foodborne illnesses. This trend reflects a growing interest in utilising natural compounds as alternatives to synthetic preservatives, especially in traditional dairy products where microbial quality is vital for consumer health and product stability.

Table 1: Total plate count of control and paneer treated with crude extract B1 and B2 (log_{10} CFU/g)

Days of storage	Control	B1	B2
0	Nd	Nd	Nd
3	Nd	Nd	Nd
6	2.85 ± 0.11^{aA}	2.60 ± 0.11^{BA}	2.28 ± 0.11^{bC}
9	3.76 ± 0.12^{aA}	3.61 ± 0.12^{aA}	2.95 ± 0.12^{bB}
12	4.57 ± 0.09^{aA}	4.35 ± 0.09^{aB}	4.00 ± 0.09^{bC}

Means bearing different superscripts between columns (a, b, c) and between rows
(A, B, C) differ significantly ($p<0.05$). *- Significant, NS- Non significant

3.4 Yeast and mould count of control and paneer dipped in different concentrations of crude extract

Table 2 presents the yeast and mould counts for the control, B1 (50% extract), and B2 (100% extract) treatments over a 12-day period. Initially, no yeast or mould growth was observed in any of the samples. By day 6, B1 recorded a minimal yeast and mould count of 0.20 ± 0.11 CFU/g, while the control and B2 samples remained free of growth. By day 9, B1's count increased to 1.10 ± 0.08 CFU/g, but the control and B2 still showed no signs of yeast or mould. By day 12, all samples exhibited detectable yeast and mould, with B2 having the lowest count at 0.51 ± 0.26 CFU/g, B1 the highest at 1.23 ± 0.26 CFU/g, and the control at 0.31 ± 0.26 CFU/g. B2 demonstrated greater effectiveness in inhibiting yeast and mould growth compared to B1, suggesting that the 100% extract offers better preservation against fungal contamination.

Table 2: Yeast and mould count of control and paneer treated with crude extract B1 and B2 (log_{10} CFU/g)

Days of storage	Control	B1	B2
0	Nd	Nd	Nd
3	Nd	Nd	Nd
6	Nd	0.20±0.11	Nd
9	Nd	1.10±0.08	Nd
12	0.31 ± 0.26^{bB}	1.23 ± 0.26^{aA}	0.51 ± 0.26^{B}

Means bearing different superscripts between columns (a, b, c) and between rows
(A, B, C) differ significantly ($P<0.05$). *- Significant, NS- Non significant

3.5 Sensory evaluation of control and paneer dipped in different concentrations of crude extract

Table 3 indicates that the mean sensory scores for control paneer and those treated with 50% (B1) and 100% (B2) crude extracts. A significant difference was observed in aftertaste, which favoured the 100% extract (B2). Microbiological analysis confirmed that B2 effectively lowered both total bacterial and yeast and mould counts compared to B1. As a result, B2 selected as the superior treatment, demonstrating its enhanced ability to improve both the sensory qualities and microbiological safety of paneer during storage. This study reinforces the growing evidence supporting the use of natural bio-preservatives in dairy products to enhance food safety and shelf life while maintaining their sensory and nutritional qualities. Mendiratta *et al*. (2007) [9] also reported a similar trend in sensory scores for control and treated samples of vacuum-packaged skim milk paneer over the storage period

Table 3: sensory evaluation of control and paneer dipped in different concentrations of crude extract

Sensory attribute	control	B1	B2	Chi-square(P-value)
Appearance and colour	8.33±0.12	7.85±0.10	7.95±0.23	4.66^{ns} (0.097)
Body and texture	8.29±0.18	7.45±0.04	7.58±0.19	5.82^{ns} (0.05)
flavour	7.66±0.12	7.29±0.11	7.29±0.07	5.64^{ns} (0.05)
Odour	7.50±0.12	7.04±0.17	7.0±0.14	2.54^{ns} (0.28)
After taste	$7.75±0.19^{A}$	$7.04±0.19^{B}$	$7.39±0.14^{A}$	7.30*(0.02)
Overall acceptability	7.91±0.16	7.41±0.12	7.58±0.08	3.27^{ns} (0.19)

Means bearing different superscripts between columns (a, b, c) and between rows (A, B, C) differ significantly (p<0.05). *- Significant, NS- Non significant

4. Conclusion

The present study concluded that the application of 100% lactic acid bacterial crude extract has a significant preservative effect on paneer. In contrast, the use of 50% crude extract resulted in higher yeast and mould counts compared to both the control and the 100% crude extract. This increase may be attributed to the higher water content in the 50% crude extract. However, it is noteworthy that the yeast and mould counts observed on the 12th day of storage for the treatment group remained within the limits set by FSSAI standards. Therefore, utilizing crude extracts from curd can effectively extend the shelf life of paneer.

Conflict of Interest

Not available

Financial Support

Not available

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